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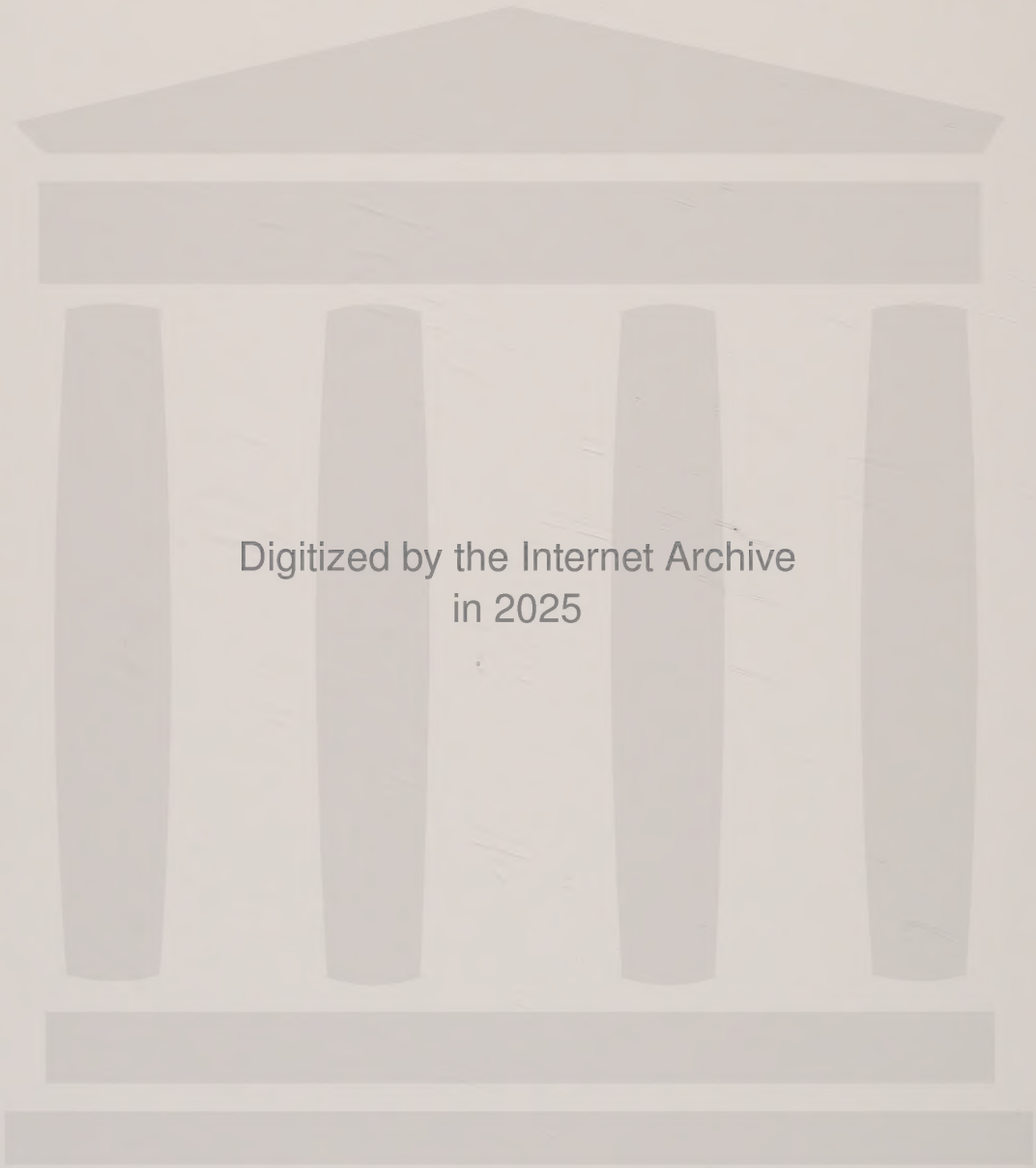
Studies of the Effects on Fish of Effluent from a Titanium Dioxide Plant

BY

J. S. EDMONDS

1981

WESTERN AUSTRALIAN
MARINE RESEARCH LABORATORIES
DEPARTMENT OF FISHERIES AND WILDLIFE
PERTH, WESTERN AUSTRALIA



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Fish. Res. Bull. West. Aust. 1981, **26**, 1–21

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STUDIES OF THE EFFECTS ON FISH OF EFFLUENT FROM A TITANIUM DIOXIDE PLANT

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ABSTRACT

The results of three sets of experiments to determine the effects on fish of effluent from a titanium dioxide plant are presented.

The acute incipient lethal concentration of the batch of effluent tested (free sulphuric acid 26·3 g/l, soluble ferrous iron 6·55 g/l) was ca 3 200 p.p.m. (21 p.p.m. iron, pH 5·1) for juvenile yellow-eye mullet (*Aldrichetta forsteri*) and ca 2 930 p.p.m. (19 p.p.m. iron, pH 5·4) for juvenile school whiting (*Sillago bassensis*). Oxidising ferrous iron had an acute incipient lethal concentration of ca 6 p.p.m. to juvenile yellow-eye mullet on first exposure. Iron lost its toxic properties as soluble ferrous iron oxidised to insoluble, flocculent ferric iron.

Juvenile yellow-eye mullet tolerated very brief (ca 60s) exposure to pH 2 but were rendered motionless for the period of exposure. For juvenile school whiting the values were pH 2·4 for ca 30s.

Fish from a variety of species tended to avoid the cloud produced by the addition of ferrous iron to seawater when the iron concentration in the cloud was 0·5 p.p.m. Experiments with clouds produced by the addition of silica to seawater indicated that the avoidance behaviour was a visual phenomenon.

Caution is advised in the extrapolation of the results of laboratory experimentation to the field situation.

I. INTRODUCTION

Since 1963 Laporte Australia Limited has been manufacturing titanium dioxide from locally mined ilmenite at Australind, Western Australia.

The sulphuric acid process employed produces waste consisting of ferrous sulphate, sulphuric acid, small amounts of some heavy metals (chromium, vanadium, zinc) and a little unchanged ilmenite. The detailed composition of the batch of effluent used in the experiments described here is shown in Table 1.

After a brief period of disposal directly into the sea, waste from the Laporte plant has been emptied into lagoons created in the calcareous sand dunes that separate Leschenault Inlet from the sea (see location map: Figure 1). It recently became evident that the capacity of the dunes to assimilate this waste is limited and saturation was near. Consequently other modes of disposing of the effluent were considered. One of the methods examined was by a pipeline extending several kilometres into Geographe Bay and linking with that from the Laporte plant crossing Leschenault Inlet (Figure 1).

Although the biological consquences of the dumping of wastes from titanium dioxide plants have been studied in some detail for certain locations in the Northern Hemisphere, e.g. New York Bight (Vaccaro *et al* 1972), Gulf of Bothnia (Lehtonen and Tuunainen 1974), the Humber Estuary (Wilson *et al* 1974), it was evident that direct extrapolation of the results of such studies to the Geographe Bay situation was inappropriate and probably misleading. Consequently a trial pipeline (50 mm in diameter) extending 5 km from shore and carrying about 3% of the total waste from the plant was positioned in 1975 and consultants were engaged to assess the biological and physical effects of waste disposal over one year of its operation.

The Department of Fisheries and Wildlife undertook to perform aquarium studies on the effects of the waste on certain of those species of fish commercially sought in the area but not forming part of the reef communities. The results of these studies are reported here.

Three series of experiments were performed:—

(i) Acute static toxicity tests.
(ii) Toxicity trials under conditions of rapidly changing pH.
(iii) Studies of the behaviour of fish in the presence of hydrated ferric oxide floc.

II. ACUTE STATIC TOXICITY TESTS

A. Introduction

Acute toxicity tests have been carried out for many substances on a few convenient fish species and general principles and procedures have been published (Sprague 1969; Portmann 1972). Limited tests on the toxicity of acid-iron wastes from titanium dioxide plants have been performed on a number of fish species. Maggi and Lassus (1974) determined the toxicity of acid-iron waste to the eel (*Anguilla anguilla*) and the stickleback (*Gasterosteus aculeatus*). Wilson *et al* (1974) reviewed the biological effects of acid-iron waste in the United Kingdom and briefly described toxicity experiments on the flatfish genus *Pleuronectes* and the armed bullhead (*Agonus cataphractus*). Toxicity studies in Finland with perch (*Perca fluviatilis*) and whitefish (*Coregonus lavaretus*) reported by Seppänen and Shemeikka (1972) are noted in reviews by Lehtonen and Tuunainen (1974) and Lehtonen (1975). Detailed work on sublethal effects of acid-iron waste on embryos and larvae of North Sea herring (*Clupea harengus*) and juveniles of the painted goby (*Gobius pictus*) and sole (*Solea solea*) have been published by Kinne and Rosenthal (1967) and Kinne and Schumann (1968).

It is evident from the above listed studies that the major short-term toxic factor (for adult fish at least) is pH rather than a metallic component of the effluent. However, of the two major chemical processes that occur on the addition of acid-iron waste to seawater, neutralisation of the acid component is, under optimal conditions, a fast reaction, whereas oxidation of ferrous to ferric iron, even after a pH greater than five has been achieved is much slower (Fig. 7). There is a possibility, therefore, that fish in water of non-lethal pH may be subjected to soluble ferrous iron in the process of oxidation. Field observations (T. D. Meagher, pers. comm.) suggested that some factor other than low pH may have been responsible for caged fish deaths close to the experimental ocean outfall. Some additional experimentation was performed in the study reported here to determine if soluble oxidising iron is of the same toxicity as oxidised flocculent iron. To stress any possible difference in toxicity between oxidised and reduced iron, toxicity tests were also carried out on solids recovered after oxidation of both total acid-iron effluent and pure ferrous sulphate, i.e. tests on fully oxidised effluent deprived of its acidic component and

the equivalent concentration of homogeneous oxidised iron.

Of the fish used in this study, the yellow-eye mullet (*Aldrichetta forsteri*) has been used previously for toxicity trials (Negilski 1976), and its use for Australia-wide evaluation of toxicity of oils and dispersants has been recommended (Negilski 1975).

B. Materials and Methods

Toxicity trials with acid-iron waste.

Apparatus: Six glass aquaria of 110 l capacity each were used. All tanks were artificially aerated by ducted air from a centralised compressor. Natural seawater pumped from the Indian Ocean close to the Research Laboratories at Waterman was used in all tests and is described as follows:—

Temperature range: for all experiments: 21·2° to 24·3°C. (The temperature range for any single trial did not vary by more than 0·4°.)

Salinity range: for all experiments: 35·28‰ to 35·82‰.

pH: 8·1 (All pH values were obtained with a Phillips Model PW 4909 digital display meter.)

Oxygen: Vigorous aeration of all test tanks ensured oxygen saturation.

Effluent: The composition of the effluent used in all toxicity trials is given in Table 1.

TABLE 1

COMPOSITION OF EFFLUENT USED IN ACUTE TOXICITY TRIALS

Free sulphuric acid	26·3 g/l
Total sulphuric acid	40·7 g/l
Soluble ferrous iron	6·55 g/l
Soluble chromium	7·0 p.p.m.
Soluble zinc	7·1 p.p.m.
Soluble vanadium	6·9 p.p.m.
Suspended solids	0·70 g/l
Soluble titanium dioxide	0·62 g/l

Test Species: Juvenile yellow-eye mullet, *Aldrichetta forsteri* (70–150 mm in length) were collected from Crawley Bay in the Swan river, and from Whitfords Beach 8 km north of the Waterman Laboratories. Juvenile school whiting, *Sillago bassensis*, (60–130 mm in length) were collected from Whitfords Beach. Collections were made by beach seine net and fish were transported to the aquarium in 100 l capacity polythene drums aerated by oxygen from a cylinder.

Experimental procedures: Fish were maintained in clean seawater and fed daily (mussels) for at least a week before being used for testing. Only healthy fish taking food regularly were used. Yellow-eye mullet and school whiting are both notable for the ease with which they may be maintained in aquaria. Making up of the test solutions was complicated by the two chemical processes occurring on addition of acid-iron waste to seawater.

The rate of oxidation of ferrous to ferric iron is pH dependant (Hem and Cropper 1959). Oxidation is very slow at pH's less than 5 but appreciable at pH's greater than 5. As a consequence of the existence of this threshold, mixtures of effluent (pH 0·75) in seawater designed to produce pH's less than 5 resulted in clear, or slightly cloudy pale green solutions containing ferrous iron. More dilute mixtures of effluent and seawater (pH's $>$ 5) were, on the other hand, able to oxidise rapidly and contained ferric iron in suspension. Therefore, unlike the majority of toxicity trials previously reported, testing acid-iron effluent was not simply a case of exposing fish to a range of different concentrations of single or mixed toxicants but involved different chemical species at different dilutions. In all cases the solutions were made up several days before addition of the fish and were vigorously aerated. The pH's of the solutions were taken daily. Those solutions with initial pH above 5 were found to increase in pH value by about 1 unit over 2–5 days. Solutions with pH less than 5 remained constant or showed a very slight drop in pH (about 0·2 pH units). Such variations were presumably due to movement in the CO_2/HCO_3^- buffer system.

Fish were transferred to the experimental tanks in soft netting. Six tanks were used, five containing effluent dilutions and the other used as a control. The six tanks were in a single horizontal line and the distribution of the different dilutions and control was random. Ten fish were placed in each tank. Lighting conditions were from the standard aquarium background; 12 hours low level fluorescent lighting and 12 hours darkness. Vigorous aeration was maintained throughout the toxicity trials. Dilutions of effluent used were: 1 effluent: 200 seawater; 1:250; 1:330; 1:340; 1:350; for the whiting. 1:100; 1:200; 1:250; 1:275; 1:300; 1:325; 1:350; for the mullet. The tanks were observed continually for the first 2 or 3 hours after the fish were added then at intervals of a few hours. Dead fish were removed.

Toxicity trials with oxidising ferrous iron

Apparatus: Six glass aquaria of 20 l capacity each containing 10 l of seawater were used. Tanks were artificially aerated by ducted air from a centralised compressor. Seawater conditions were as described above for the static toxicity tests.

Test Species: Juvenile yellow-eye mullet (70–150 mm in length) collected from Whitfords Beach were used in this series of experiments.

Experimental procedures: A stock solution of hydrated ferrous sulphate (Ajax Chemicals, analytical grade reagent) in seawater (64 g/l) was prepared containing a trace of hydrochloric acid to retard oxidation. Fifty ml of this solution (containing 650 mg iron) was added to each of five of the six aquaria. These tanks thus contained iron at a concentration of 65 mg/l. The sixth tank served as a control and contained only seawater. Fish (7) were added to each of the tanks a predetermined time after the addition of ferrous iron. Two series of experiments were carried out. Vigorous aeration was sufficient to maintain all insoluble iron in suspension. Sizes of floc particles were measured with a Nikon Profile Projector, Model 6C (Nippon Kogaku K.K.).

The rate of oxidation and precipitation of the iron was demonstrated by the vigorous aeration of a tank of seawater containing, initially, ferrous iron at 640 p.p.m. Samples were taken from the tank for the determination of soluble (ferrous) and insoluble (ferric) iron at intervals of 5 min initially and subsequently at 30 min as oxidation neared completion. The samples were filtered (millipore, 1·2 μm) and the iron in filtrate and filtrand determined by atomic absorption spectrophotometry.

Toxicity trials with recovered solids:

Hydrated ferric oxide together with other solids present in the oxidised effluent were obtained by the addition of the waste to seawater under conditions of vigorous aeration. The pH was maintained above 5.

After oxidation was judged to be complete the aeration was stopped and the floc allowed to settle. The bulk of the supernatant liquid was removed by decantation and the solids filtered from the remainder. The solids were washed 3 times with distilled water and dried at 50°C. Chemically homogeneous hydrated ferric oxide was obtained by the oxidation of aqueous solutions of ferrous sulphate.

Both the impure hydrated ferric oxide derived from the effluent and the homogeneous material from chemical preparation were used to prepare suspensions at concentrations equivalent to a 1:100 dilution of effluent to seawater (65·5 mg/l Fe). Tests on these suspensions were carried out in 20 l capacity glass aquaria using batches of seven fish per tank. A control tank containing clean seawater was used for each trial.

Statistical treatment

Results were analysed by the log-probit method developed for pharmacological purposes by Bliss (1937) and since modified and extended to fish toxicity testing (Litchfield 1949; Fry *et al* 1946). Use of this method has been recommended by Sprague (1969) and is described at length by Burton *et al* (1972). The method involved the plotting of mortality data on log-probability paper and the fitting of straight lines by eye. The time for death of 50% of fish (ET_{50}) was read off for each concentration.

Secondly a toxicity curve was constructed from which the incipient lethal concentration was apparent. This was done in this study by using logarithmic scales for both time (ET_{50}) and toxicant concentration (Sprague 1969; Wilson 1972). Curves were fitted by eye.

C. Results

Toxicity trials with acid iron waste

Log-probability plots of mortality versus time are shown in Figure 2 (mullet) and Figure 3 (whiting). Plots of log ET_{50} against log concentration are shown in Figure 4 for both species. It can be seen that the acute incipient lethal concentration of the batch of effluent tested was *ca* 3 200 p.p.m. for yellow-eye mullet and *ca* 2 930 p.p.m. for school whiting. The corresponding pH values were 5·1 for mullet and 5·4 for whiting. Effluent in sea water dilutions of 1:325 and 1:350 (3 077 and 2 857 p.p.m.) for mullet and 1:350 (3 857 p.p.m.) for whiting failed to produce any mortality over the duration of the experiments (192 h.).

Toxicity trials with oxidising ferrous iron

Log-probability plots of mortality versus time are shown in Figure 5. A plot of log ET_{50} versus log soluble iron concentration at the start of each trial is shown in Figure 6. It is apparent that iron at the concentration used (65 mg/l) lost its toxicity as soluble ferrous iron oxidised to insoluble, flocculent ferric iron. Those fish killed, died with their gills closed and mouths partly open. Much red/brown flocculent material had accumulated in the gill filaments and it is possible that oxidation and precipitation within the gills caused death. There was no evidence of increasing particle size of hydrated ferric oxide with time, i.e. water samples taken just after oxidation was initiated contained floc of the same particle size as after oxidation for 1 hour, (the only difference being the greater quantity in the latter case).

No mortalities were recorded in control tanks.

The ratio of oxidation and precipitation of the iron measured both by the appearance of insoluble ferric iron and the disappearance of soluble ferrous iron is shown in Figure 7.

Toxicity trials with recovered solids

No mortalities were recorded in the tanks containing hydrated ferric oxide and total effluent solids equivalent to dilutions of 1 of effluent to 100 of seawater (65·5 mg/l Fe). No mortalities were recorded in control tanks.

III TOXICITY TRIALS UNDER CONDITIONS OF RAPIDLY CHANGING pH

A. Introduction

Little work has been carried out on the acute effects of lowered pH on marine organisms apart from studies intended to define the short term toxic factor in acid-iron effluent (Wilson *et al* 1974; Maggi and Lassus 1974). Most studies of pH effects on fish, for example that of Mount (1973) on the growth and reproduction of the fathead minnow (*Pimephales promelas*), relate to freshwater with its greater pH range particularly on the acid side of neutrality. Such studies are of little relevance to the marine situation. Carter (1964), however, reported on the survival of fish (*Gobius paganellus, Pleuronectes platessa, Pleuronectes flesus, Mugil chelo, Morone labrax*) in acidic seawater. He found a pH within the range of 5·0–5·5 to be critical for each of those species. Above pH 5·5 survival was prolonged in all cases to greater than 96 hours.

The object of the experiments reported here was to discover if fish could survive, and how they would react to, a short exposure to low pH water. It was thought that fish swimming into or through a plume of acidic water might be so exposed. Clearly the severity of the effects on a fish of an immersion of short duration in low pH water will affect its ability to swim to clean water.

B. Materials and Methods

Apparatus: A glass aquarium (600 mm x 600 mm x 450 mm) of 162 l capacity was used. A system of perforated P.V.C. pipes throughout the tank allowed the entry of either acid water (dilute sulphuric acid) or clean seawater. Two stopcocks controlled their flow. The low pH water was contained in a header tank 1m above the experimental aquarium. Clean seawater entered from the aquarium header tank 2 m above the experimental aquarium. The apparatus is shown in Figure 8. A 100 mm width strip of nylon mesh was placed around the lip of the tank to prevent fish from being swept out when the dilution water was flowing. The header tank for low pH water contained 300 l of dilute sulphuric acid at about pH 1·5 (1·4–1·8). This corresponded to a molarity of sulphuric acid of 0·023 (0·030–0·013). The relationship between molarity of sulphuric acid in seawater and pH is shown in Figure 9. The drop in pH in the experimental tank when the stopcock allowing entry of dilute sulphuric acid was open, and the rise in pH when this stopcock was shut and the other allowing entry of clean seawater opened, is shown in Figure 10. Samples for pH determination were taken by 100 ml capacity syringe. It was shown by taking samples from corner locations after 60 seconds that the pH throughout the tank at that time was uniform within narrow limits. It was assumed that this uniformity persisted throughout the experiments.

Water temperatures ranged from 19·6°C to 22·5°C over all experiments and salinities from 35·14‰ to 35·82‰. However, because of the short duration of each trial negligible variation was experienced within each one.

Test Species: As for acute static toxicity tests.

Experimental procedures: The following procedures were followed for each experimental run:

(a) Five fish (mullet or whiting) were placed in the experimental tank filled with clean aerated seawater. The pH of the seawater was recorded.

(b) 300 l of dilute sulphuric acid at a precisely measured pH of about 1·5 were placed in the header tank.

(c) After deciding the lowest pH required and the time the fish were to be exposed to that pH, the stopcock allowing entry of dilute sulphuric acid was opened and left open for the appropriate time. It was then closed and after the pre-decided time interval the stopcock allowing entry of clean seawater opened. A water sample for pH determination was taken at the time of closing the dilute sulphuric acid stopcock.

(d) When sufficient time had elapsed for the pH to have risen to that of normal seawater, the fish were gently removed in soft nylon net and placed in "post treatment" tanks for observation.

(e) Those surviving were kept for two weeks before being released.

(f) It was assumed that the pH course of the addition of sulphuric acid and the subsequent dilution with seawater would be the same as the appropriate sections of the calibration curve shown in Figure 10.

C. Results

The pH courses (pH *v* time) of the trials are shown in Figure 11 (mullet) and Figure 12 (whiting). The mortalities resulting from the exposures described by these plots and the lowest pH achieved are recorded in the figures also. In all cases as the pH was lowered the fish became very active and frequently broke the water surface. As the pH was reduced and/or the time of exposure increased the fish became inactive and floated or lay on the bottom of the tank with ventral surface upper-most. It has been suggested (Carter 1964) that such behaviour may be attributed to the release of carbon dioxide within the water as well as to lowered pH. As the pH was increased the fish regained apparently normal activity. Some fish did not right themselves and resume normal behaviour until after some minutes in the "post treatment" tanks. It was notable that those fish killed appeared to die on or immediately after exposure to acid water. If still alive on resuming clean water virtually all fish recovered, even those whose only sign of life at such times was slight gill movement. Fish were judged dead when gill movement had ceased for 5 minutes. Those fish killed died with their mouths wide open and gills ajar.

IV STUDIES ON THE BEHAVIOUR OF FISH IN THE PRESENCE OF HYDRATED FERRIC OXIDE FLOC

A. Introduction

Aquarium experimentation on the avoidance reactions of fish to foreign substances in the water has been described by Sprague (1969) who reviewed some earlier work. Most studies present the fish with a choice of clean or contaminated water and the time spent by the fish in each water body is recorded. Apparatus for similar tests was first devised by Jones (1947) and clearly such experimentation is readily susceptible to simple statistical analysis.

For the experiments described here an annular tank was used and a suspension of the iron floc added at a single position in the surface water. As migratory fish and those obtaining food by fast and continual movement were under study, it was thought that an annular tank would most satisfactorily allow for directional swimming. The addition of the floc at a single position and its gradual diffusion from that position would most closely simulate the conditions likely to be encountered in the sea disposal area. It was hoped that fish would either make even use of the available space after addition of the ferric oxide floc, or would show some bias in choice of water quality. However, the schooling nature and, in some cases, low numbers of fish used favoured a purely descriptive approach and this has been employed in the results section relating to this experimentation.

In an attempt to relate aquarium experiments to the field situation, turbidity measurements were made with a transmissometer type turbidity meter in the area of sea around the end of the effluent pipe. Some information on background turbidity (admittedly on a single day) was obtained and further experiments were carried out in the annular tank with the seawater rendered turbid (by the addition of finely divided silica) to the same degree as in the field, before addition of the iron floc.

In addition, to discover if avoidance by fish of iron floc added to the seawater was a general reaction to a light occlusive material or was a response to a property possessed by iron floc alone, experiments were carried out with finely divided silica added into the water surface (clean, filtered seawater) in the same manner as the iron floc in other experiments.

Although no previous aquarium trials on the behaviour of fish exposed to ferric oxide floc have been reported, Lehtonen and Tuunainen (1974) describe the effects of acid-iron effluent, in particular the resulting hydrated ferric oxide, on the distribution of fish in the Baltic Sea:

> The precipitated iron has also been found to have a clear expelling effect on fish when it has risen to the surface as a result of strong winds and upwelling. According to the observations made by fishermen, Baltic herring and whitefish avoid these colour clouds and fishing gear set in the way of such clouds may give good catches before the cloud but in the turbid areas the catches are very small if the fish are caught at all.

B. Materials and Methods

Field turbidity measurements

Field turbidity measurements in Geographe Bay were made with a Kahlsico No. 269WA100 turbidity meter (Kahl Scientific Instrument Corporation, California) operated from the deck of R.V. *Flinders*. The meter was calibrated in distilled water (100% transmission). The full optical path of 1 m was used for maximum sensitivity. Readings were taken at the intersection points of a half sea-mile grid (Figure 13a–d) in the surface water and at each half fathom until the instrument struck the seabed.

Fish behaviour experiments

Apparatus: An annular concrete tank of 3 m diameter outer ring, 1·5 m diameter inner ring and 0·75 m in height was constructed with a horizontal ladder walkway 2·5 m above the tank and spanning it on a diameter. One hundred mm width baffles were placed in pairs attached to the inner and outer walls of the tank to aid division of the tank into 6 segments and to help prevent the formation of annular currents which might have caused too rapid mixing of the ferric oxide floc.

The observer, concealed in an asbestos box situated on the walkway, was able to record the positions of the fish during the course of the experiments without disturbing the fish. Shade cloth covered the tank and observer position in an attempt to even out lighting conditions. The tank was located in an outside courtyard.

The tank is shown in plan in Figure 14. The hydrated ferric oxide (made by oxidation of ferrous sulphate; Ajax Chemicals, analytical grade reagent) or silica suspension and equivalent volumes of seawater as controls were added into the surface water of the tank by the trough mechanisms shown in Figure 15. Each device consisted of a trough with closed ends pivoting about its long axis between the inner and outer walls of the annulus. The two troughs (one for iron floc or silica, the other for the seawater control) were situated on opposite sides of the tank.

A cord was attached to the underside of each trough and when pulled by the concealed observer caused the trough to invert. The levels of test material and seawater in the troughs were carefully adjusted to be the same as the water in the tank. Thus inversion of a trough was equivalent to removing the barrier between its contents and the water in the tank. There was virtually no mechanical disturbance of the water.

Test Species: Juvenile yellow-eye mullet and school whiting were obtained as under acute toxicity testing above. Australian herring, *Arripis georgianus* (Whitford's Beach and Cockburn Sound), juvenile tailor *Pomatomus saltatrix* (Claremont), and a single specimen of spotted whiting, *Sillaginodes punctatus* (Cockburn Sound) were obtained from the locations indicated in parentheses. All fish were transported to the laboratory in 100 l capacity polythene drums of oxygenated seawater. All fish were maintained for at least two weeks before being transferred to the experimental tank. Some were maintained for several months before being used.

Experimental Procedures: the fish under test (always of one species) were transferred to the experimental tank by soft net and allowed to acclimate for at least 24 hours. Although, by the use of shade cloth, attempts were made to produce even lighting there were patches of comparative light and shade within the tank, and all fish tended to accumulate in the shaded area.

Consequently the trough used for addition of the hydrated ferric oxide floc or silica was located to add its contents into the shaded area. In that way attempts to avoid the test materials would mean acting against their instincts to seek a preferred light intensity. All tests were carried out at the same time of day in conditions ranging from heavy overcast to clear sunny weather. After filling the troughs with the appropriate solutions the observer climbed into the observation box and remained concealed for 30 minutes before pulling the cords and inverting the troughs. The positions and behaviour of the fish were observed and recorded.

The hydrated ferric oxide was added into the surface water *via* the trough mechanisms at concentrations chosen to produce suspensions containing 0·125–0·5 p.p.m. iron (i.e. floc derived from 3, 6, 12 g $FeSO_4 . 7H_2O$) if evenly distributed throughout the total volume of the tank (4 764 l). Silica was added into the surface water, *via* the trough mechanism, at a concentration designed to replicate the light occlusive properties of iron floc derived from 6 g hydrated ferrous sulphate (16·5 g silica). This value was decided by using the Kahlsico turbidity meter to take readings of the light transmission through hydrated ferric oxide suspensions of a range of concentrations. The same optical path (1m) and calibration (distilled water = 100%) was used as in field measurements. A plot was made (Figure 16) of % transmission *v* concentration of iron (p.p.m.). In order to determine the quantity of silica producing the same light occlusion as hydrated ferric oxide floc containing iron at 1 p.p.m., silica was added from a weighed stock until the desired % transmission was achieved. It can be seen from Figure 16 that 1 p.p.m. iron is equivalent to 70% transmission. The concentration of silica reducing the transparency of filtered seawater from 100% to 70% was 13·6 p.p.m. As the relationship between iron concentration and % light transmission is virtually linear for the region of interest (Figure 16), it was assumed that the relationship between silica concentration and light transmission would be linear in the same manner.

Silica used for increasing background turbidity and for avoidance experiments was silica gel H for thin layer chromatography (E. Merck, Darmstad). The particle size of this preparation was similar to that of the iron floc.

When the turbid cloud produced by 16·5g of silica occupied half of the tank it was equivalent to a cloud containing 0·5 p.p.m. iron. The weight of iron floc produced by 6 g hydrated ferrous sulphate was 2·3 g (calculated as $Fe(OH)_3$). 2·3 g silica was added into the surface water in a further trial. Thus the *weight* of material in the water was replicated rather than the opacity. By this means it was hoped to demonstrate whether fish were responding to optical or chemical properties of the materials in suspension.

In a further trial the whole tank was rendered turbid by the addition of 33 g silica. This replicated in terms of opacity, an iron cloud containing 0·5 p.p.m. iron. This, in turn, is equivalent to a turbidity meter measurement of 87% (Figure 4). It can be seen from Figures 13a–d that field turbidity measurements around the end of the pipeline were (October 7, 1976) *ca* 75–87% depending on depth. Fish were acclimated to the turbid water for 12 hours. Iron floc produced by 6 g hydrated ferrous sulphate was added into the surface water *via* the trough mechanism.

Water temperatures and salinities for each trial are given in the results section.

C. Results

Hydrated ferric oxide floc alone

Australian Herring

The reactions of all species of fish tested were similar. The behaviour of a small school of herring (8 fish) is given below in detail as an example. Observations on the fish 5 days prior to testing are given initially.

Date: June 4, 1976;

Weather: Overcast, no wind;

Water temperature: 18·1°C;

Salinity: 35·01‰.

Time: 9.30 All 8 fish were grouped in a single segment of the tank offering most shade.

9.40 The school had moved about 0·5 m but were still in the darkest area.

9.50–10.20 As above.

Date: June 8, 1976;

Weather: Fine, no cloud, no wind;

Water temperature: 18·0°C;
Salinity: 34·99‰.

Time: 9.40 All 8 fish were grouped in a segment adjacent to that occupied during June 4, 1976 but still in shaded area.

9.50–10.20 As above

11.15 Hydrated ferric oxide floc formed from dissolution and oxidation of 12 g hydrated ferrous sulphate was added in 3 l of seawater from the trough directly over the school of herring. Three l of seawater were added from the opposite trough. (12 g ferrous sulphate would produce an iron concentration of 0·5 p.p.m. if evenly distributed throughout the tank). The fish were slightly disturbed when the trough emptied and moved to the edge of the shaded area but did not enter the sunlit zone.

11.22 The hydrated ferric oxide cloud occupied 1 segment (one sixth) of the tank. The school of fish moved to and fro in the small area of tank between the iron cloud and the sunlight.

11.24 The hydrated ferric oxide cloud occupied almost 3 segments (a half tank). The fish were caught and stationary in a very small area between the iron cloud and sunlight.

11.25 The hydrated ferric oxide cloud occupied a little over half the tank. The fish avoided it to the extent of passing through the sunlight into another small area of shadow.

11.35 The hydrated ferric oxide cloud filled four fifths of the tank and started to enter the small area of shadow occupied by the fish. The fish were in, and did not leave, the only part of the tank not affected by the cloud.

11.45 The flocculent cloud occupied the entire tank and the fish were unable to avoid it. They showed more movement than normal when exposed to it.

There appeared, until the cloud filled the whole tank, to be a sharp dividing line between the cloud and clear water, i.e. there did not appear to be a concentration gradient from clear water to maximum density at the point of application. If it is assumed that the concentration of hydrated ferric oxide was constant throughout the cloud at any given time, then when the cloud occupied half the tank, iron was at a concentration of 1 p.p.m., and when it occupied the whole tank, iron was present at 0·5 p.p.m. Herring therefore, possess the ability to detect and avoid hydrated ferric oxide with iron at a concentration of 0·5 p.p.m.

School whiting

Three trials were held with schools consisting of 5 fish each. Final concentration (when the hydrated ferric oxide cloud was evenly distributed throughout the whole tank) were 0·5, 0·25 and 0·125 p.p.m. of iron

Whiting showed the same ability to detect and avoid the hydrated ferric oxide cloud as did herring when it was present at 0·5 p.p.m. iron. 0·5 p.p.m. iron did, however, appear to be about the threshold, either for detection by the fish or for an avoidance response to occur. The whiting avoided the cloud produced by 6 g ferrous sulphate until it occupied over half the tank. If it is assumed that the hydrated ferric oxide was evenly distributed within the cloud, this again suggests a threshold of about 0·5 p.p.m. iron. The whiting similarly first entered the cloud of hydrated ferric oxide produced by 3 g of ferrous sulphate when it occupied a little over one quarter of the tank. Again 0·5 p.p.m. iron would result from even distribution of this cloud. On one occasion whiting entered the cloud (occupying a little over half the tank and resulting from 6 g ferrous sulphate) and were seen to turn back when approaching the boundary between the cloud and clear water. The school remained in the cloud and oscillated between the two interfaces of turbid and clear water for some minutes. There was thus some evidence that it was the interface between the two water bodies of different quality rather than the water quality itself that repelled the fish.

Other species

Yellow-eye mullet and tailor behaved very similarly to Australian herring and whiting. Both were tested with the addition of hydrated ferric oxide from 6 g ferrous sulphate and both were first seen to freely enter and leave the cloud only when it occupied about half the tank or more. The spotted whiting, a large specimen, entered and left the cloud when it occupied about one quarter of the tank, i.e. an iron concentration of about 1 p.p.m.

Silica

Australian herring were used for these experiments. Silica suspension (16·5 g in seawater) produced an even white cloud which spread at a similar rate to the iron cloud. In three separate trials schools of herring (9, 6 and 5 fish) avoided the cloud until it occupied slightly more than one half of the tank. After some apparently uncertain movements near to the cloud boundary they entered and left the cloudy region. 2·3 g silica added into the surface water produced avoidance behaviour only when it was a discrete cloud occupying less than half of one segment of the tank. This was for a very short time only.

Floc added into water made turbid with silica

Australian herring in two separate trials (schools of 5 and 6 fish) showed the same avoidance of iron floc (derived from 6 g hydrated ferrous sulphate) superimposed on water rendered turbid with silica (33 g per tank: equivalent to 87% turbidity meter reading) as in experiments where the same quantity of iron floc was added into clear, filtered seawater i.e. when the iron cloud occupied approximately one half of the tank (0·5 p.p.m. iron if evenly distributed).

In all cases where avoidance behaviour was observed the fish turned back from the cloud (iron or silica) some 10–20 cm before the boundary between clear and turbid water.

V. DISCUSSION

The acute toxicity trials reported here suggest, because of the non-lethal nature of oxidised effluent deprived of its acidic component, that pH is the major critical property in the short term. This observation is in agreement with those reported by Wilson *et al* (1974) and Maggi and Lassus (1974). These workers report little difference in toxicity between certain concentrations of effluent from titanium dioxide plants and an equivalent concentration of sulphuric acid. However, experiments reported here have shown that oxidising solutions of

ferrous iron are more toxic than the equivalent suspension of oxidised ferric iron. Because oxidation occurs at a much slower rate than neutralisation, it is possible that the toxic nature of oxidising ferrous iron may be of greater importance to fish in the field than low pH.

The behavioural experiments indicate that avoidance by fish of flocculent material is a visual phenomenon (at least in the daylight intensities in which the experiments were performed) and is dependent only on the opacity to light of the suspension of test material.

Presumably such a behavioural reaction would occur during daylight hours only. Confirmation of the visual nature of the response could come from experimentation at low light intensities or with fish rendered sightless. It is interesting that fish already subjected to a background turbidity similar to that encountered in the sea area of the pipeline did not show diminished response to added iron floc.

The extrapolation of results from aquarium experiments to the field situation is problematical. It is especially so in this case as currently the field situation is not clearly defined or understood. However, it is possible that there would be three quite different areas within the disposal zone of the effluent, each producing a different effect on fish. Firstly there is likely to be quite a small area (small if water movements and other factors favour rapid mixing and dilution) of low pH water acutely toxic to fish. Secondly there is likely to be a larger area of normal pH but oxidising ferrous iron possibly lethal to fish, and thirdly a much larger area (irregularly shaped by prevailing currents and wind conditions) containing dispersed hydrated ferric oxide floc that may have considerable effects on fish behaviour and movement (possibly during daylight hours only). With regard to the latter zone, it must be stressed that extrapolation from aquarium behaviour experiments to the field is likely to be less tenable than from experiments involving mainly physiological responses. The boundaries of these areas can be identified under varying field conditions by direct observation.

It is unlikely that adult migratory fish will be killed by the effluent because it seems that they would be discouraged from approaching close enough to the outfall to be in the region of acute toxicity. However, the work of Kinne and Rosenthal (1967) on the effects of a very diluted effluent from a German titanium dioxide plant (virtually hydrated ferric oxide floc) on the eggs and larvae of North Sea herring suggests that affected regions of Geographe Bay serving as fish nurseries may be in some danger. The importance of Geographe Bay as a fish nursery is yet to be firmly established. The immediate environs of the outfall are likely to be more important in this regard than more exposed areas further south but less important than shallower, sheltered, inshore waters (R. C. J. Lenanton, pers. comm.)

It has been shown that juvenile yellow-eye mullet and school whiting can survive brief exposures to water at pH's as low as 2 but their behaviour is considerably affected. It is doubtful whether, in the field situation, fish would be allowed to lie motionless and recuperate from such pH shock. However such effects must be described as "acute" and if dilution and mixing is adequate are likely to be confined to a small sea area.

VI ACKNOWLEDGEMENTS

Mr. J. A. Stoddart provided valuable help with practical aspects of this work. In particular he designed the mechanism for adding test materials to the tank in the behaviour study. Mr. K. A. Francesconi carried out the iron analyses. Messrs. I. L. Lethbridge and C. R. Bell prepared the figures. Thanks are due to Dr. D. A. Hancock for helpful suggestions and a critical review of the manuscript.

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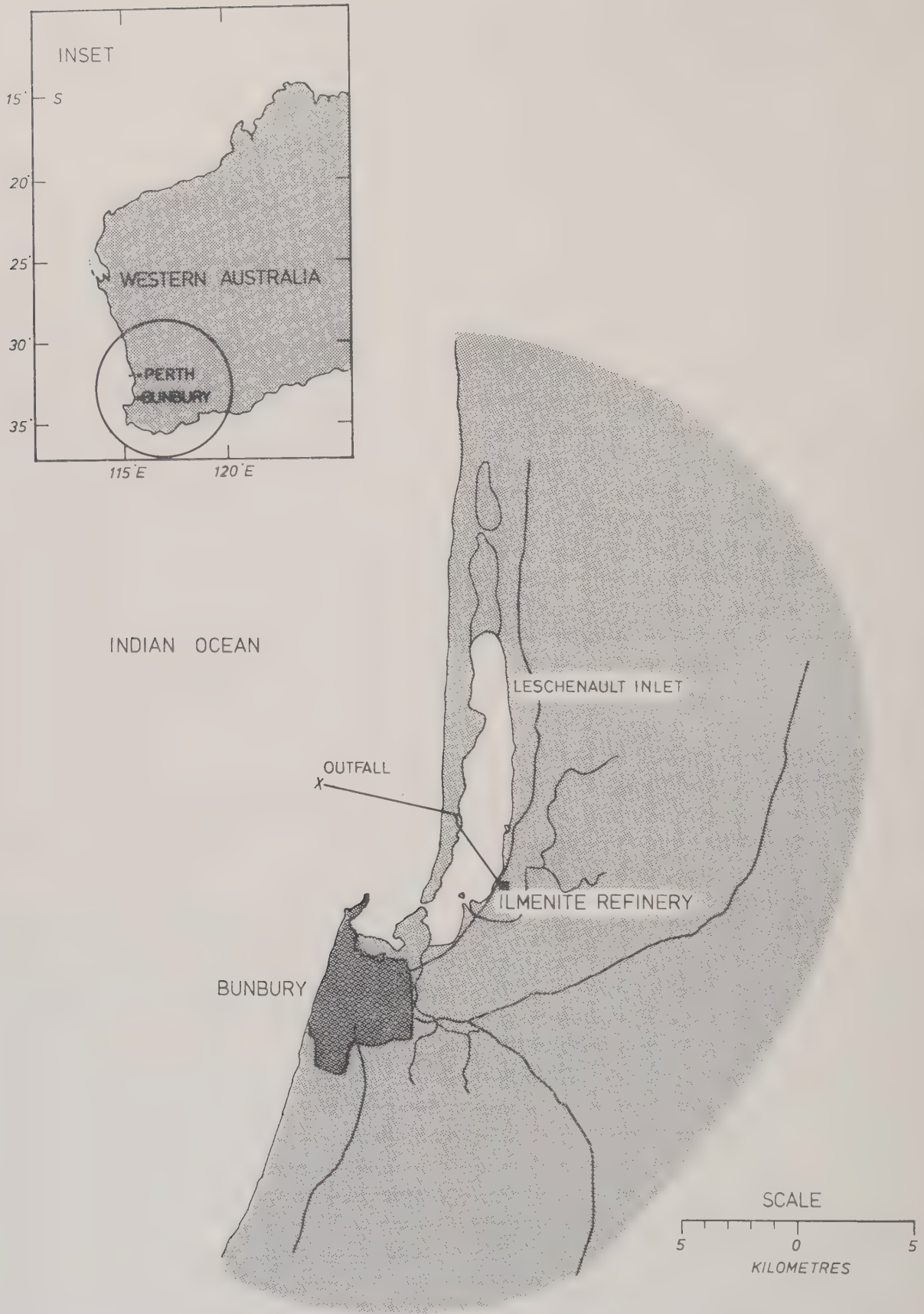


Figure 1—Map showing location of the Laporte ilmenite refinery and ocean outfall.

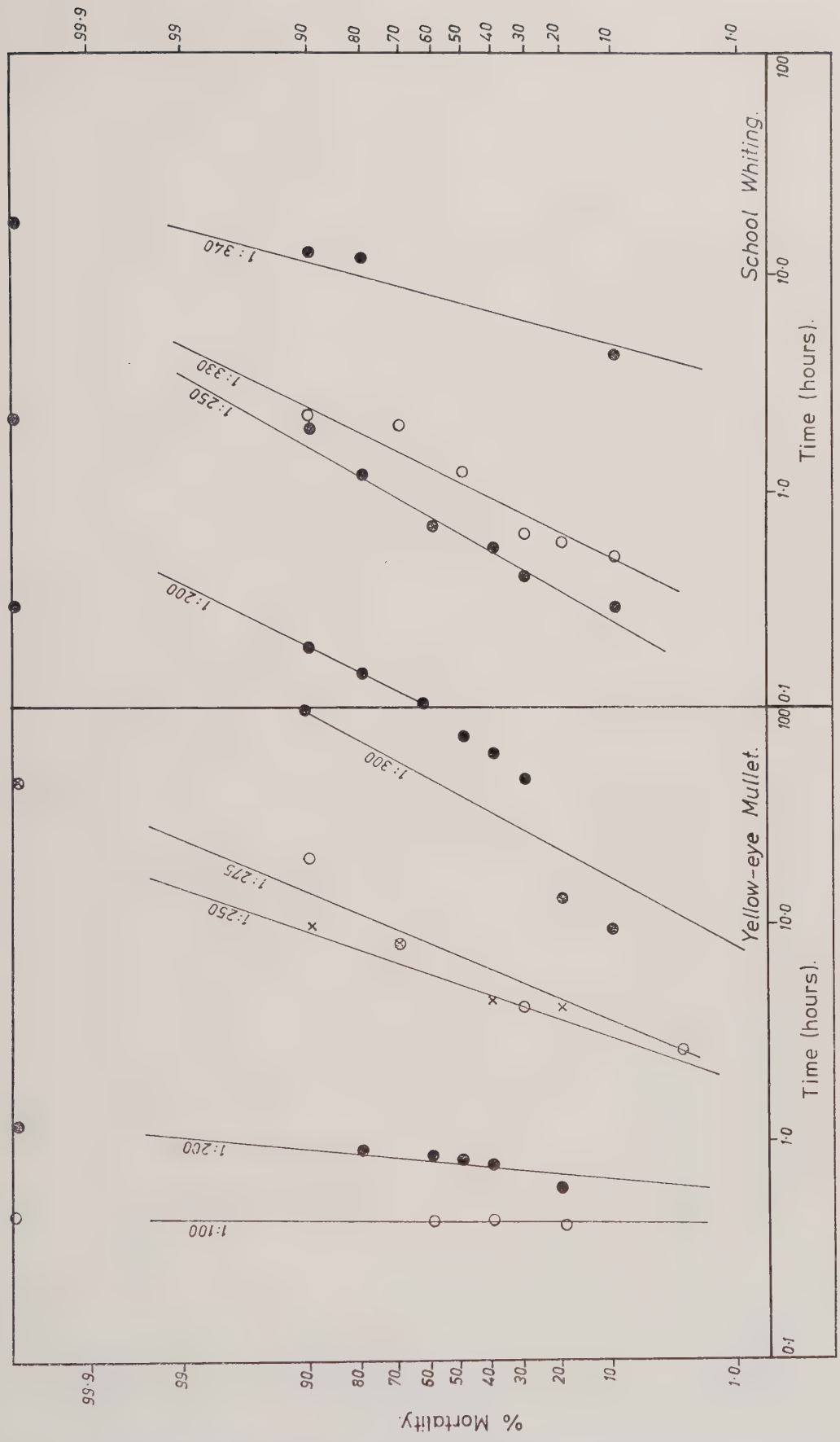


Figure 2

Figure 3

Figures 2 and 3—% mortality *v* time plots for yellow-eye mullet and school whiting.

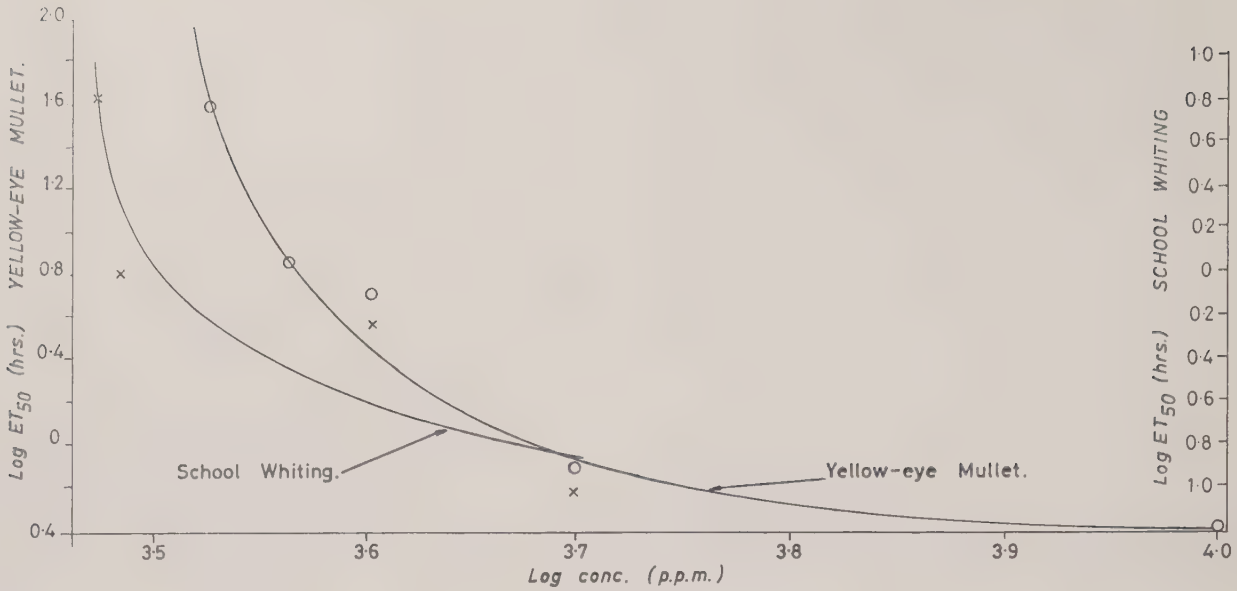


Figure 4—Log. ET_{50} *v* Log. effluent concentration for yellow-eye mullet and school whiting.

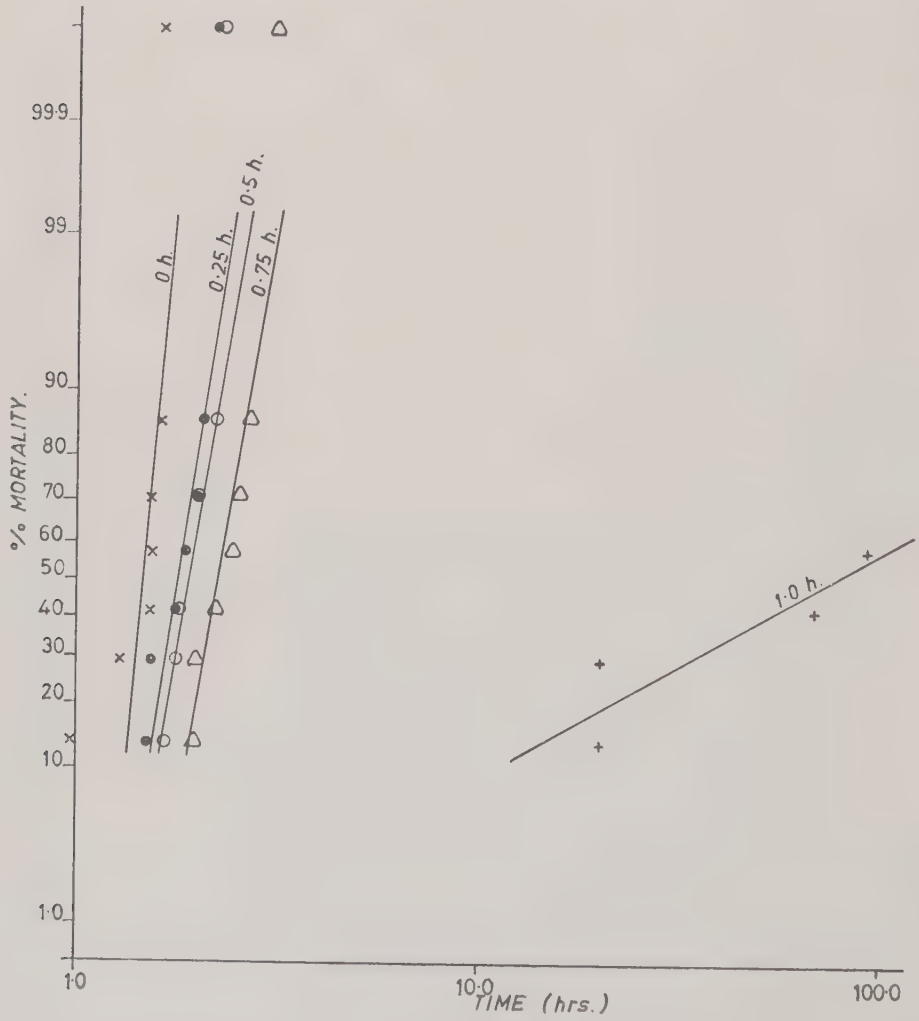


Figure 5—Oxidising iron: % mortality *v* time plots for yellow-eye mullet.

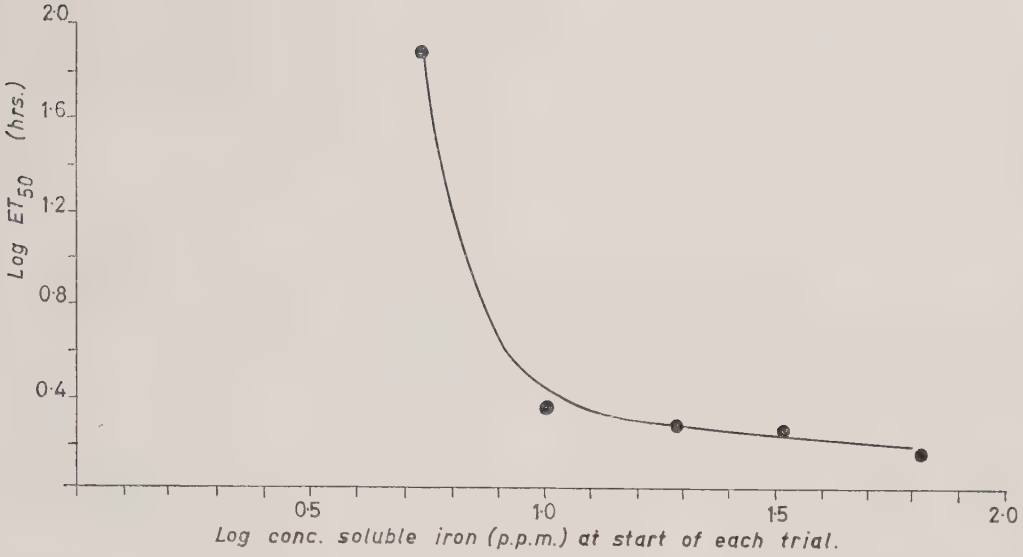


Figure 6—Oxidising iron: Log. ET_{50} *v* Log. concentration of soluble iron at the start of each trial—yellow-eye mullet

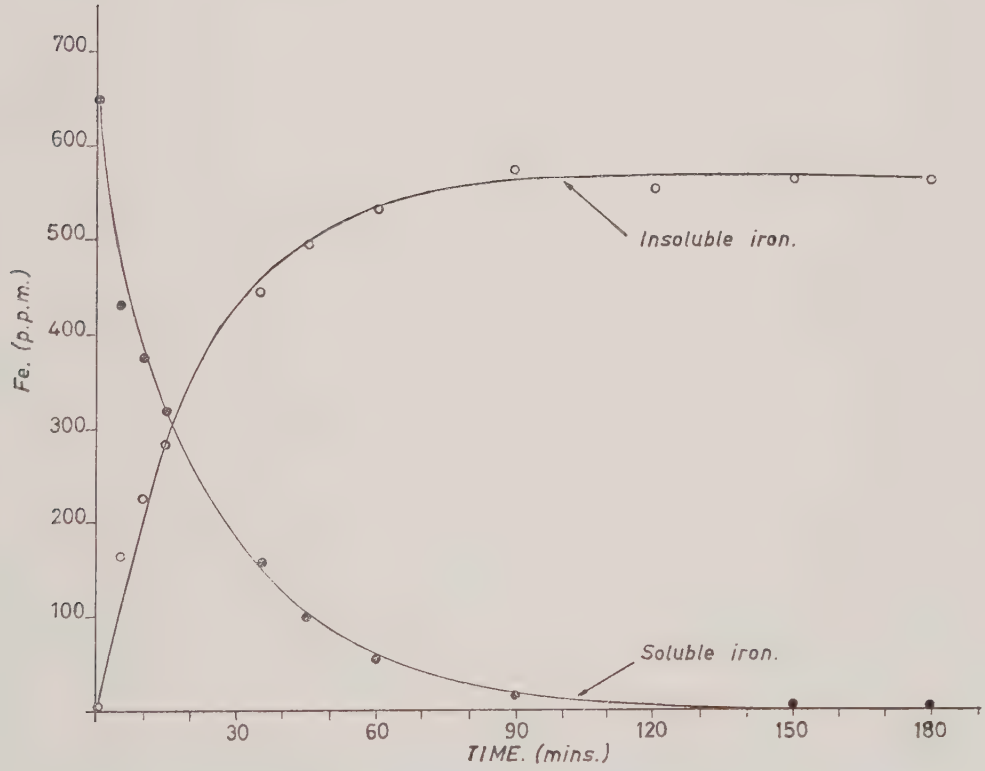


Figure 7—Oxidation of Fe^{++} (soluble) to Fe^{+++} (insoluble) in aerated seawater.

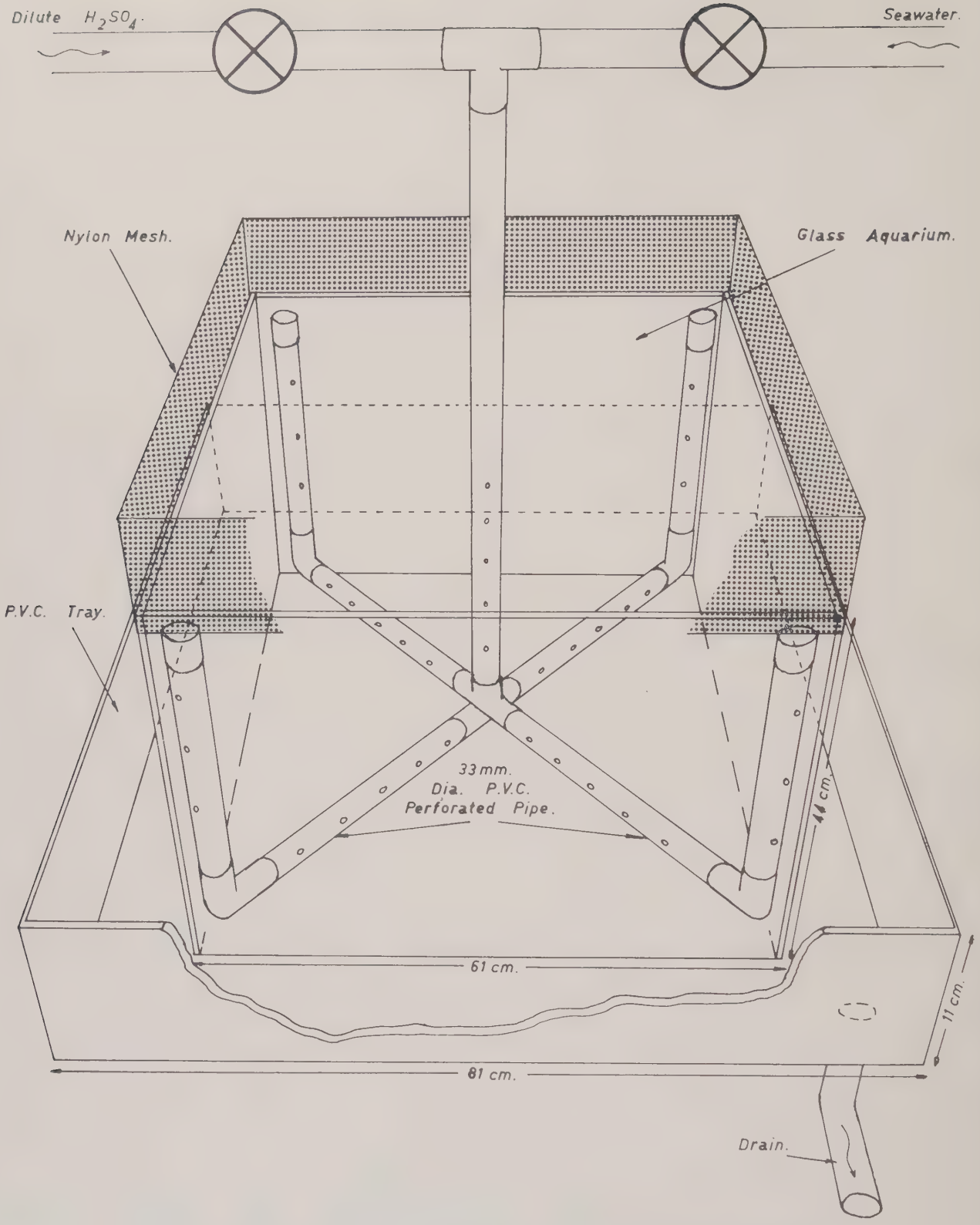


Figure 8—Tank used for toxicity trials under conditions of rapidly changing pH.

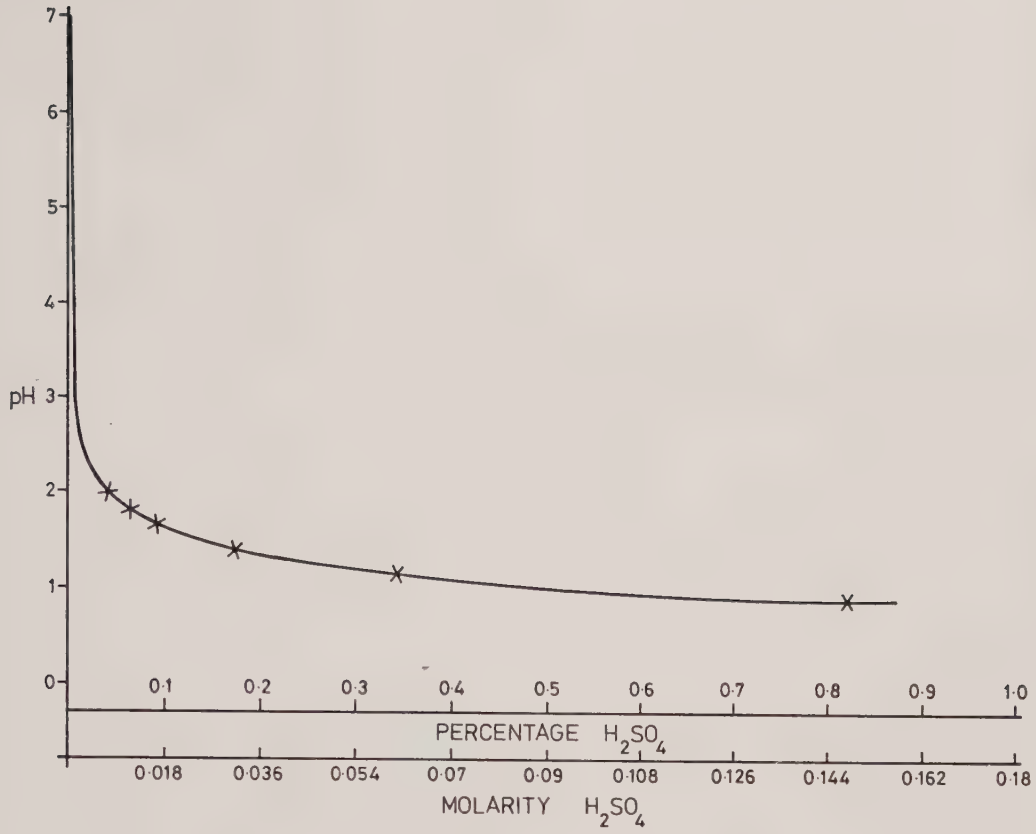


Figure 9—pH *v* percentage and molarity of sulphuric acid.

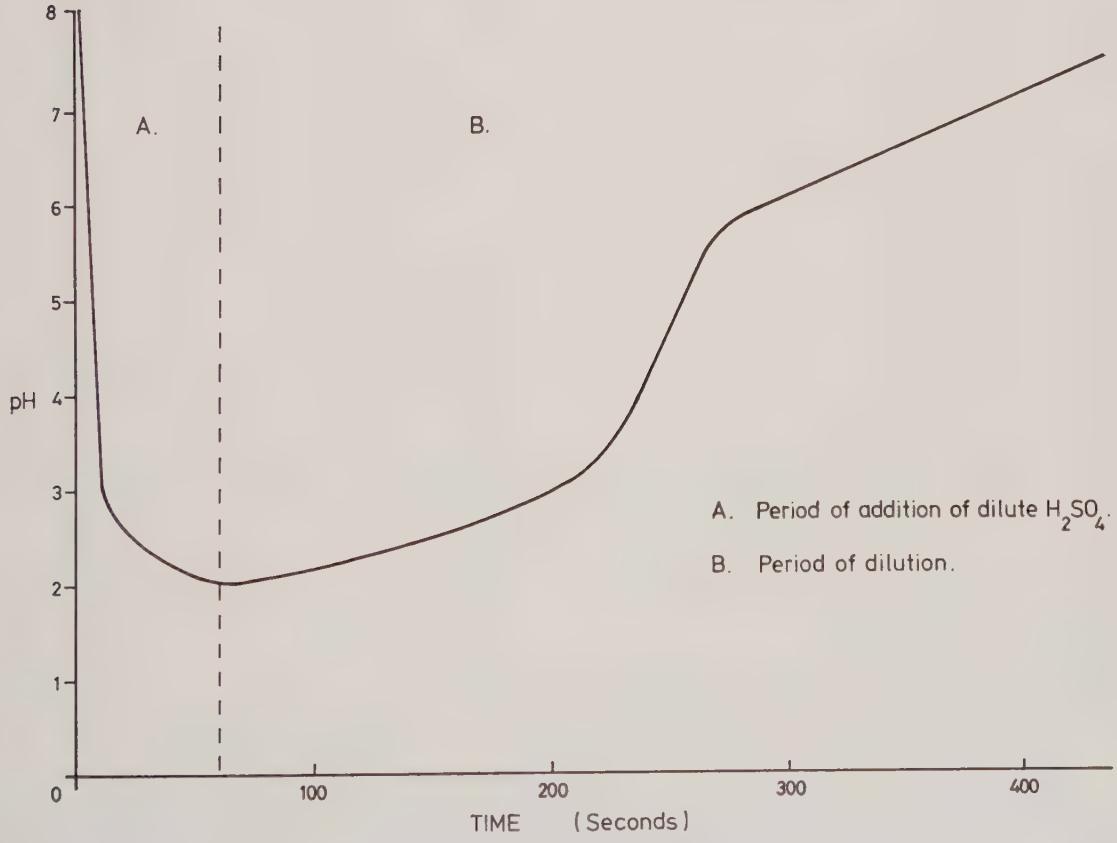


Figure 10—pH profile of the addition of dilute sulphuric acid to seawater and its subsequent further dilution with seawater.

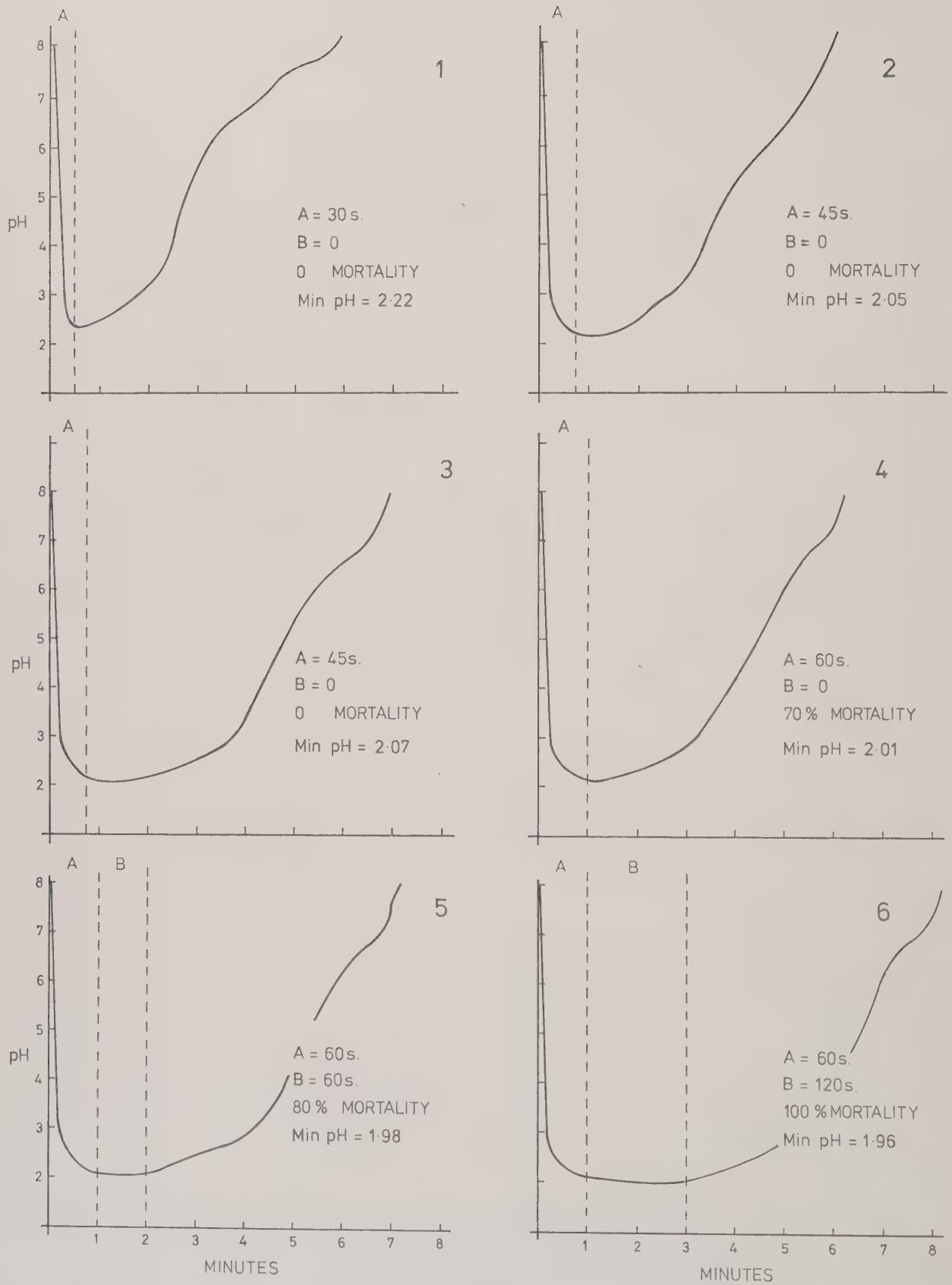


Figure 11—Experimental pH profiles: yellow-eye mullet. A = Period of addition of dilute H_2SO_4. B = Period after addition of acid and before dilution.

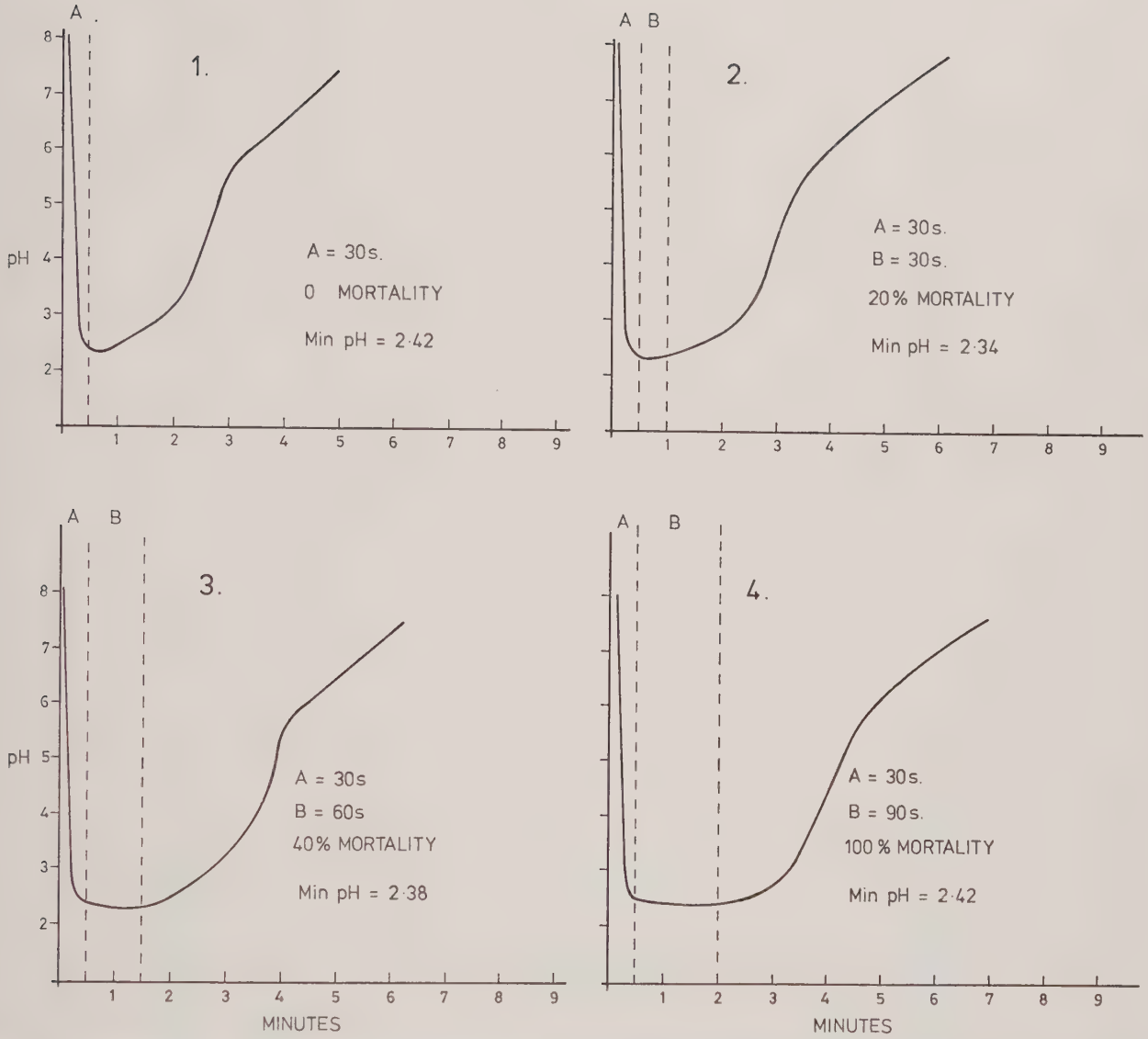


Figure 12—Experimental pH profiles: school whiting. A = Period of addition of dilute H_2SO_4. B = Period after addition of acid and before dilution.

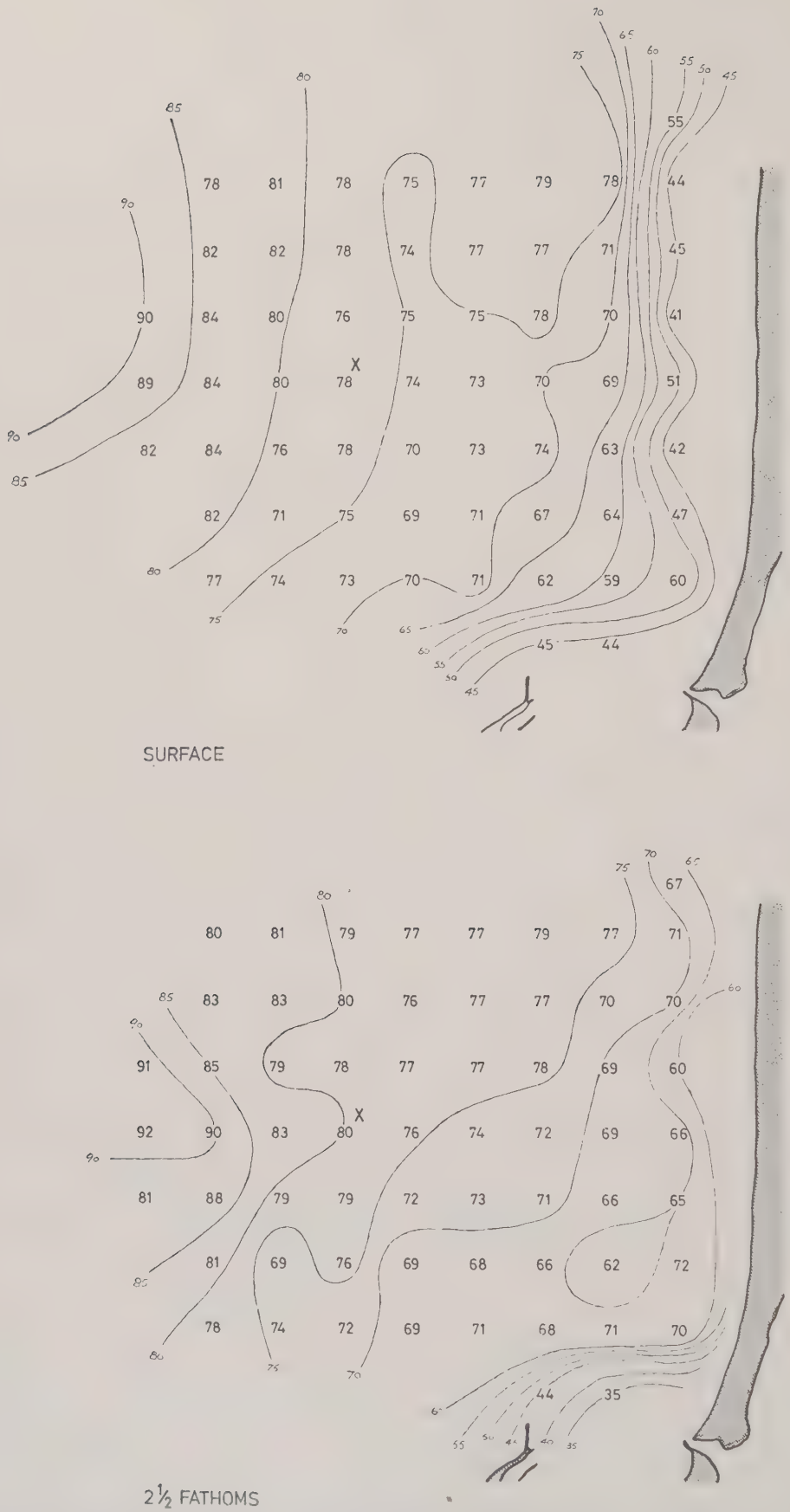
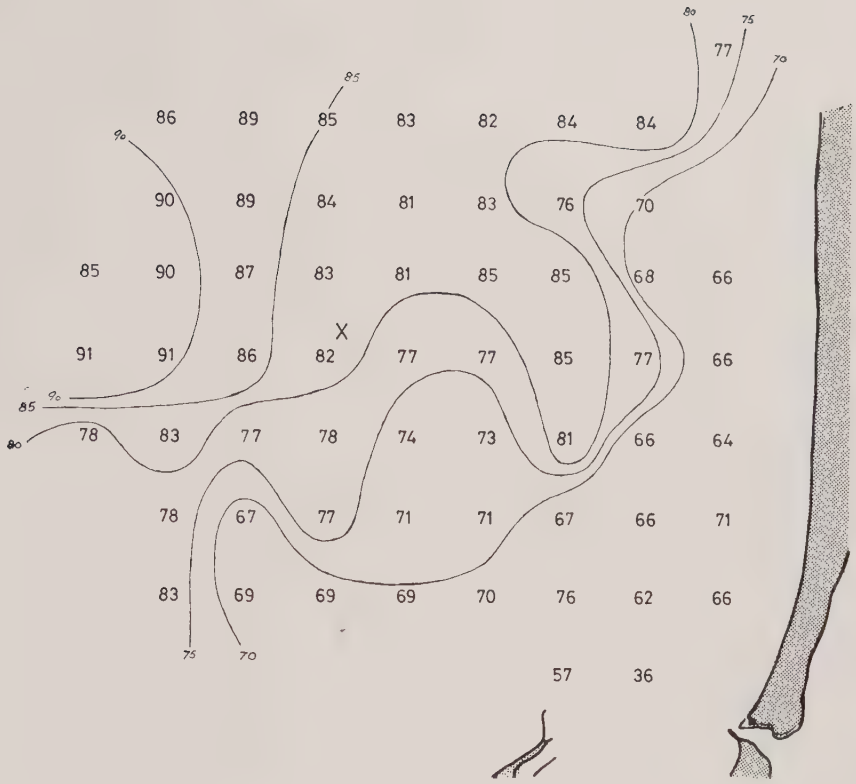
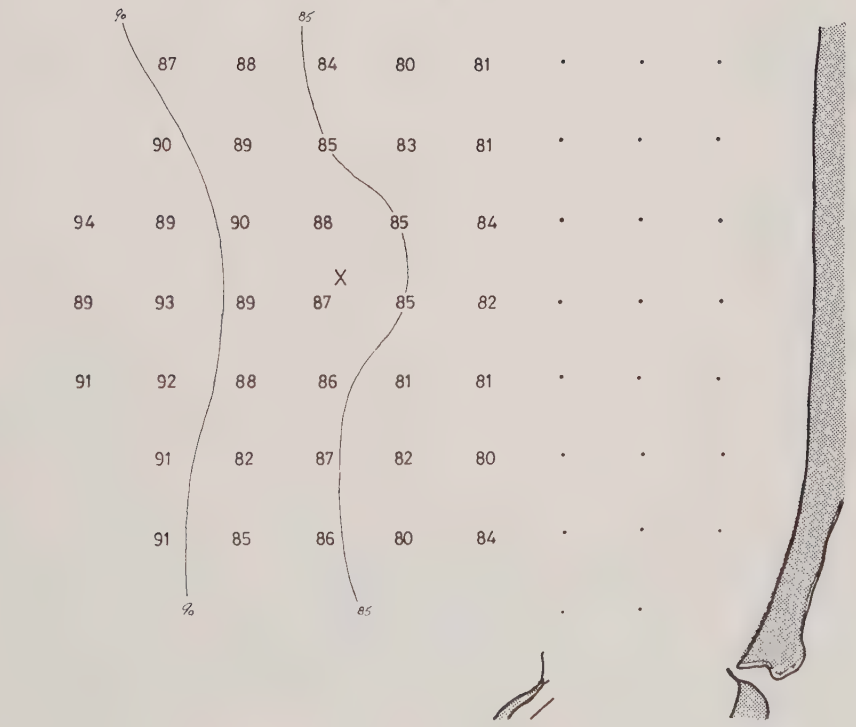


Figure 13—Turbidity in effluent disposal area. Figures are for % transparency. (Distilled water = 100%.) The location of the outfall is indicated by "X".



5 FATHOMS



7½ FATHOMS

Figure 13—*continued.*

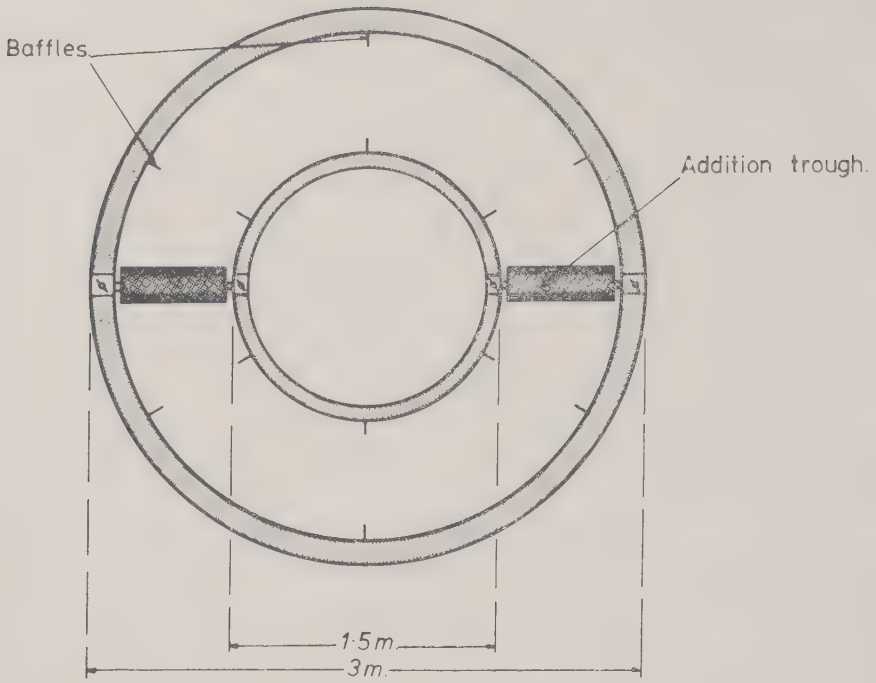


Figure 14—Annular tank—Plan.

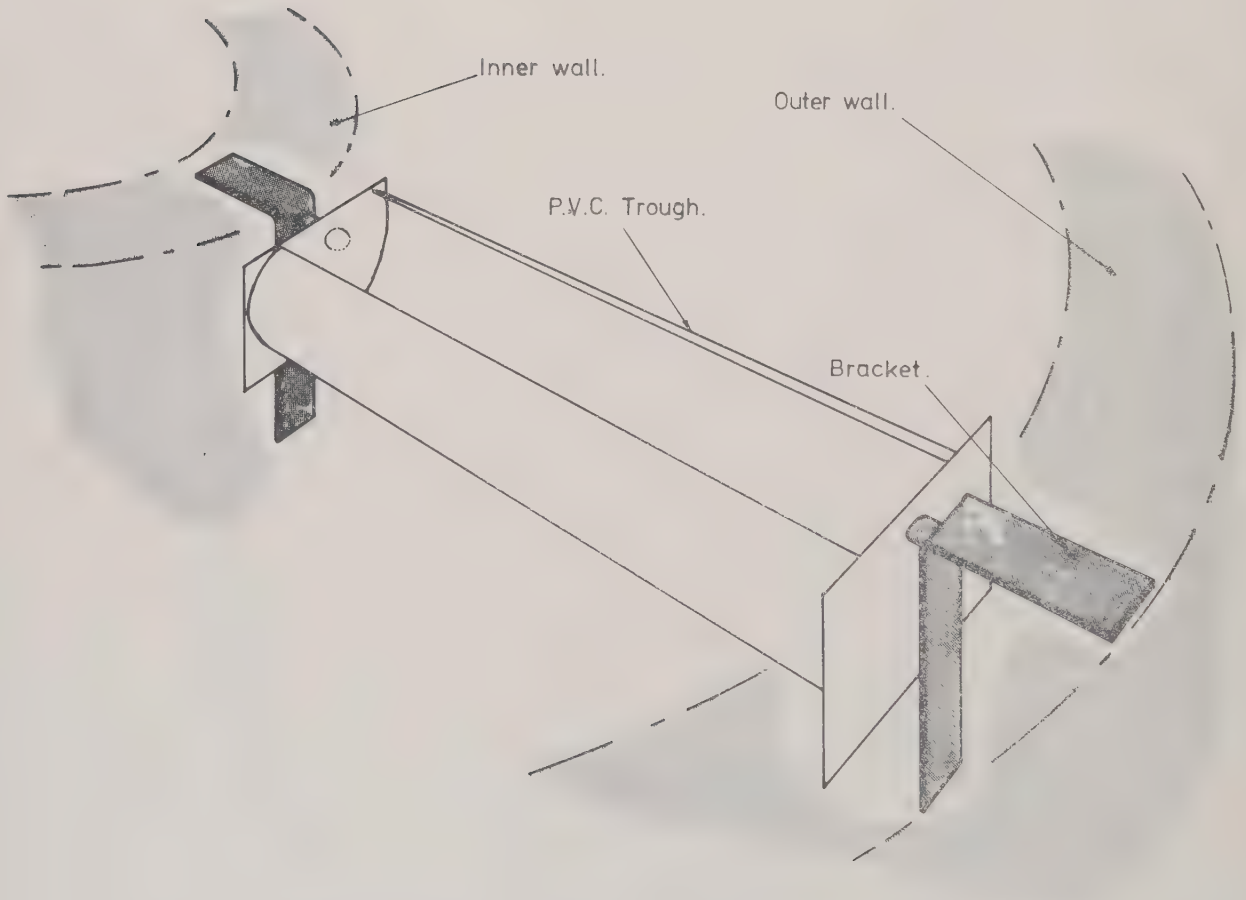


Figure 15—Trough for addition of test materials to tank.

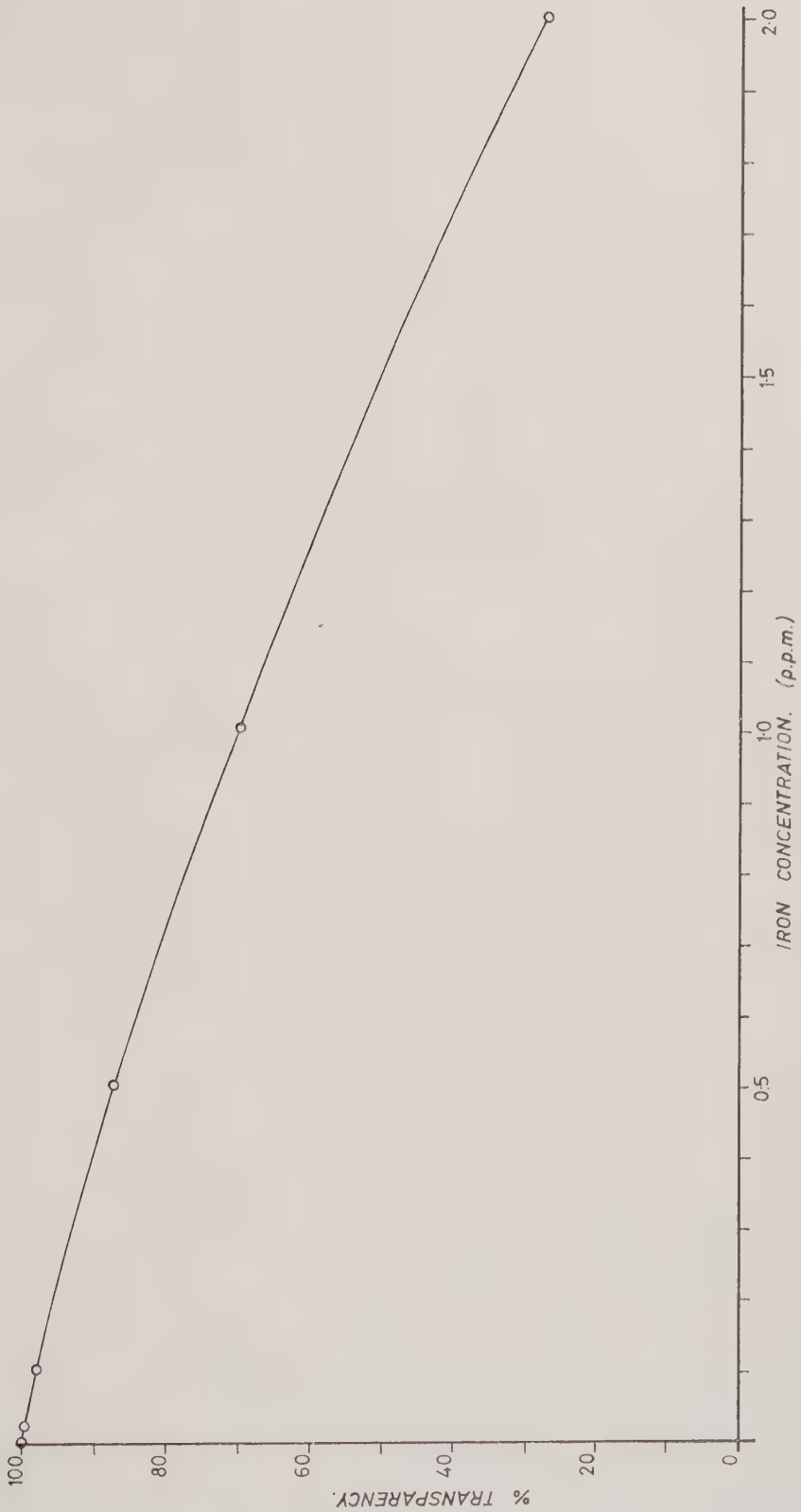


Figure 16—Percentage transparency *v* iron concentration in seawater. Kahlsico No. 269WA100 turbidity meter.

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